human vaccines. After several passages and plaque purifications, two Vero-adapted high growth influenza H5N1 vaccine viruses (Vero-15 and Vero-16) have been selected and could reach high virus titer (10^8 TCID50/ml) in Vero cells in T flasks. After tested with NIBRG-14 standard antiserum provided by the NIBSC, antigenicity of the Vero-15 and Vero-16 viruses remain similar to the NIBRG-14 virus. In addition, the Vero-15 and Vero-16 viruses do not have any nucleotide difference in HA and NA gene segments compared with the NIBRG-14. For process development, Vero cells were furture cultured on cytodex 1 microcarriers in spinner flasks. Vero cells growed to 3×10^6 cells/ml with a seeding density of 4.4×10^5 cells/ml in 5 g/L microcarriers and peak virus titers reached 10^9 TCID50/ml. In conclusion, the Vero-15 and Vero-16 viruses are suitable for production of influenza H5N1 vaccines in Vero cells. Their other six internal gene segments could also be used to generate vaccine seed viruses of other influenza subtypes for production in Vero cells.

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43.008

Induction of IFN-γ and IL-17 by Pneumococcal Surface Protein C-Based Vaccines does not Protect Against a *Streptococcus pneumoniae* Invasive Challenge

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Streptococcus pneumoniae (pneumococcus) is an important pathogen that causes pneumonia, meningitis and otitis media. Invasive diseases usually follow colonization of the respiratory tract and may be favored by factors that cause immunosupression. Several pneumococcal proteins actively participate of these events playing different roles such as facilitating bacterial adhesion to epithelial cells or evasion from the immune system. Pneumococcal Surface protein C (PspC) is a virulence factor that has been implicated both in colonization and in invasive phases of pneumococcal diseases. Antigen delivery systems based on live recombinant lactic acid bacteria represents a promising strategy for mucosal vaccination, since they are able to elicit both systemic and mucosal immune responses. In the present work, we have evaluated the immune response and the protective activity of nasal vaccines composed of recombinant PspC (rPspC) or *Lactobacillus casei* expressing PspC (L.c.PspC). Nasal immunization of mice with both formulations did not elicit the production of anti-PspC IgG or IgA. On the other hand, ELISPOT and cytokine ELISA analysis of cultures obtained from mice 13 h after intranasal challenge with a virulent pneumococcal strain, showed an increase in IFN-γ secretion in lung cells from mice immunized with L.c.PspC and to a lesser extent, rPspC. This cytokine was also produced by spleen cells from mice immunized with both formulations. Production of IL-17 by lung cells was observed in the group immunized with rPspC whereas immunization with L.c.PspC induced the production of this cytokine only by spleen cells. IL-17 has already been implicated in cytokine or IFN-γ by our vaccines did not confer protection against an invasive challenge with pneumococci. Further studies will be necessary for the evaluation of protection against nasal colonization.

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43.009

DNA Vaccine Construct in the Presence of EV71 IRES Elicited Higher Neutralizing Antibody Titre

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Since 1997, large epidemics of EV71 infection have been reported in East and Southeast Asia. The virus has caused numbers of outbreak and infection associated with fatal neurological complications, however no vaccine nor antiviral against EV71 are available. In our study, we have developed DNA-based vaccines against EV71. The vaccines consist of structural protein VP1 of human enterovirus 71 (EV71) as fusion proteins with enhanced green fluorescent protein (EGFP), with and without the presence of internal ribosome entry site (IRES) at the 5′-end; IRESVP1/EGFP and VP1/EGFP. Expressions of both constructs were evaluated in vitro using Vero and SK-N-MC cells, and later in vivo in murine model. Evaluation of in vitro expression showed that the VP1 gene expressed by 5′UTR-VP1/EGFP is higher in comparison to construct without IRES; VP1/EGFP, in both Vero and SK-N-MC cells. The ability of the constructed DNA vaccines in eliciting immune responses were evaluated in vivo using murine model. The mice were immunized with 2 dosages of DNA vaccine followed by experimental challenge. In vivo evaluation showed that the mice group immunized with IRESVP1/EGFP confer a higher neutralizing antibody titer in comparison to the VP1/EGFP. Results from our study not only demonstrate the potential of VP1-based DNA vaccine but also suggests the feasibility of using IRES to generate better protective immunity in mice against EV71 and the possibility to develop safe vaccine against enterovirus infection.

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43.010

Protective Immunity Induced by Baculovirus-Expressed Rabies Glycoprotein and Recombinant Adenovirus Expressing Its Protein

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Background: Since rabies virus (RV) infection is fatal for both human and animals, the protective immunization by vaccines is of critical importance for disease control and prevention. Several recombinant protein and live viruses of rabies have been constructed and tested for their

immunological effects. In this study, we have produced the recombinant rabies glycoprotein (rGP) from recombinant baculovirus-infected insect cells and recombinant adenovirus expressing rabies glycoprotein (rAdV). We also investigated immunity induced by those protein and virus in experimental mice.

Methods: For construction of rGP and rAdV, G gene from field isolate (SKRRD9901PJ) was altered to replace with the codons preferred in insect cell and mammalian cell for its high-level expression. In baculovirus expression system, baculovirus DNA including genes of chaperones such as heat shock proteins was used in order to prevent aggregation of rGP and elevate its solubility. The replication-defective adenovirus expressing rabies glycoprotein was created by homologous recombinant in HEK 293 cell using human adenovirus serotype 5 DNA deleted the early transcribed E1 and E3 genes. Five female ICR mice were immunized two times in a 30-day interval with 0.4 mg of insect cell lysate including rGP given intramuscularly. Groups of five female mice were immunized with ten-fold serially diluted rAdV (10^7—10^4 $TCID_{50}$) given intramuscularly and 10^8—10^6 $TCID_{50}$ titer given orally. Mice were periodically bled under anaesthesia by retro-orbital puncture. Virus-neutralizing antibodies (VNA) were determined with CVS-11 virus on BHK-21 cells as FAVNT method and commercial ELISA (Bio-Rad).

Results and conclusion: While the VNA titers by single inoculation of rGP were low and did not continue long in existence, titers by booster injection exceeded the 0.5 IU by 180 days. However, these immunities were inferior to those by commercial inactivated vaccines. All mice immunized intramuscularly with low titer of rAdV developed high VNA within 7—14 days after one inoculation. In experiment of oral immunization of rAdV, although titer of VNA varied in individual mice, geometric mean titers showed dose dependent. VNA titers of above 2.5 IU could be elicited after oral immunization by rAdV with titer of 10^6 $TCID_{50}$ and those antibodies were lasted by 180 days without decline of titers. As a conclusion, rAdV induced high titers of rabies VNA compared to rGP and was suitable as material to induce protective immunity against rabies.

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Antibiotics - Gram Positive (Poster Presentation)

44.001

The Antimicrobial Resistance of Streptococcus pneumonia by E-test Method

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Pneumococcus is among most common gram-positive bacteria causing infection in human. Unfortunately resistance of Pneumococcus is increasing daily like other bacteria. Considering that no useful research regarding rate of resistance of Pneumococcus against ceftriaxone has been done by E-test method (at least in Isfahan province), the above study can be a base for future studies about determination of increase or decrease in Pneumococcus resistance rate.

Method: This cross-sectional prospective study was performed in 1385 on 98 *Streptococcus pneumonia* samples separated from clinical samples of patients presenting to Al-Zahra Hospital, and then MIC (Minimal Inhibitory Consentration) of antibiotics ceftriaxone and penicillin on the organisms was determined using E-Test method. Quality control was done using Pneumococcus ATCC 49619. After editing and entering in to computer, data were analyzed using SPSS-13 and WHONET-5.

Results: This study was performed on 98 patients with age range between 5 to 10 years. Among patients, 47% are female and 53% are male. The studied samples are 55% from throat, 20% from CSF, 16.5% from blood, 3% from pleural fluid, 3% from ear (patients with otitis), 1% (1 person) from abscess and 1% (1 person) from wound. Separated Pneumococci showed about 30% sensitivity to penicillin whereas MIC of ceftriaxone for cases other than meningitis is about 90% sensitivity and this MIC for meningitis considering lesser penetration of the drug in CNS, is 81.5% sensitivity.

Conclusion: Penicillin is not an effective drug for coverage of Pneumococcus even in children, but considering effectiveness of ceftriaxone, this drug alone is sufficient in cases suspicious of Pneumococcus at early presentation and vancomycin is not needed before culture result and antibiogram.

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44.002

Epidemiology of Pneumococcal Infection and Antimicrobial Resistance of S. pneumoniae Iso-Lates Gained from Adults with and Without Low Immune Status (Far East of Russia, 2003—2006)

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Despite of the gained success in diagnostics and treatment, pneumococcal infection is still remaining the leading cause if pneumonias, meningitis etc in different groups of population, including groups with different risk factors such as age, immunodeficiency, chronical somatic diseases. On data of the previous research projects of pneumococcal infections in our city, the incidence of pneumococcal pneumonias in adults over 18 years is 36 per 100 000 of population. The aim of our research was to study epidemiology of the pneumococcal pneumonias in patients with low immune status and without any clinical immune disturbances.

Methods: we studied 140 isolates of S.pneumoniae gained from patients with pneumococcal pneumonias at the age of 18—40 years, without any others somatic or immune complications (group 1), and 65 isolates gained from patients of 18—72 years with hematology diseases (myeloma, leucosis, etc); antimicrobial resistance was studied on NCCLS standards with disk-diffusion and microdiltion methods; there were performed serotyping and PFGE.

Results: there were revealed only (0/6, 15%) of strains resistant to penicillin (group 1 vs.group 2); 34,2%/56, 9% strains resistant to tetracycline, 15, 7%/24, 6% strains resistant to erythromycin, 15%/18,4% resistant to levofloxacin, 55, 7%/40, 3% resistant to co-trimoxazole and 11,4%/12, 3%